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Trophic interactions and benthic animal community structure in the Colorado River, Arizona, U.S.A.

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SUMMARY

1. *Cladophora glomerata* is the dominant filamentous green alga in the tailwaters of the Colorado River, U.S.A., below Glen Canyon Dam, but becomes co-dominant with filamentous cyanobacteria, *Oscillatoria* spp., below the confluence of the Paria River (26 km below the dam) where suspended sediments are elevated.
2. Benthic algal assemblages played an important role in the distribution of the amphipod, *Gammarus lacustris*, in the dam-controlled Colorado River through Grand Canyon National Park, Arizona. *Cladophora* and *G. lacustris* showed a weak positive relationship at ten cobble–riffle habitats in the Colorado River from Lees Ferry (25 km below the dam) to Diamond Creek (362 km downstream), while no relationship was found between *Oscillatoria* and *G. lacustris*.
3. The relationship between algal substrata and *G. lacustris* was tested by a series of *in situ* habitat choice experiments. *G. lacustris* showed a significant preference for *Cladophora* (with epiphytes) over *Oscillatoria* spp., detritus and gravel in treatment pans at Lees Ferry.
4. Epiphytic diatoms (i.e. food) were the overriding determinant of subtratum choice by *G. lacustris* in laboratory experiments. *Gammarus* chose the *Cladophora*/epiphytic diatom community over sonicated *Cladophora* with few diatoms. The amphipods also chose string soaked in diatom extract over string without diatom extract.
5. Importance of mutualistic interactions in aquatic benthic community structure is discussed.

Introduction

Factors that influence habitat selection of amphipods in marine and freshwater ecosystems are diverse but commonly relate to food and shelter. Adequate refugia are critical for these highly abundant, often relatively large and slow moving herbivores (Duffy, 1990). Host–plant specialization by amphipods based on secondary plant metabolites that defend animals from predation by fish is well documented in marine ecosystems (Hay, Duffy & Fenical, 1987, 1990; Hay *et al.*, 1988; Paul & Van Alstyne, 1988; Duffy & Hay, 1991), and to some extent in freshwater ecosystems (Newman, Kerfoot & Hanscon, 1990). Both spatial and structural components of plant architecture influence habitat selection by marine amphipods (Leber, 1985; Hacker & Steneck, 1990). Several authors (Price *et al.*, 1980; Strong, Lawton & Southwood, 1984) have reviewed similarities between terrestrial plant–herbivore interactions and marine amphipod herbivores.

Amphipods play a significant role in nutrient cycling and energy flow in streams, from leaf pack decomposition through consumption of associated fungi and bacteria (Barlocher & Kendrick, 1973; Marchant & Hynes, 1981). Diet and feeding strategies vary widely among amphipods. Diet includes fungi (Barlocher & Kendrick, 1973; Willoughby & Sutcliffe, 1976; Willoughby & Earnshaw, 1982), bacteria (Grimm & Fisher, 1989), detritus (Nilsson, 1974), nanoplankton (Blinn & Johnson, 1982), invertebrates (Moore, 1977), macrophytic algae (Deksbakh & Sokolova, 1965; Hudon, 1983), and epiphytic diatoms (Moore, 1975, 1977; Blinn, Stevens & Shannon, 1992; Pinney, 1992). Diet selection by amphipods is typically considered a function of body size and taxon-specific feeding mechanisms (Hudon, 1983).

Macroalgae provide herbivores with food, either directly or indirectly (Gregory, 1983), and provide a high surface area for attachment of filter feeders, predators and grazers (Minshall, 1984). Filamentous algae alter microhabitat conditions by modifying current, blocking light, altering dissolved oxygen, and collecting detritus (Dodds, 1991a); such changes influence macroinvertebrates on a taxon-specific basis (Towns, 1981). Mutualisms between aquatic herbivores and macroalgae may exist (Dodds, 1991b), with herbivores removing sediments and light-limiting epiphytes and providing soluble nutrients to filamentous algae like *Cladophora glomerata* (L.) Kütz.

This investigation describes the importance of *C. glomerata* and associated epiphytic diatoms to the distribution of *Gammarus lacustris* Sars in the dam-controlled Colorado River through Grand Canyon, U.S.A. We found a weak positive correlation between *G. lacustris* standing stock and *C. glomerata* biomass. *Oscillatoria* spp. and detritus are also common substrata in the Colorado River. However, no correlation has been found between biomass of *G. lacustris* and biomass of these substrata. Field and laboratory experiments were conducted to test the relationship between *G. lacustris* and various substrata in the Colorado River.

Materials and Methods

The distributions of *Cladophora glomerata, Oscillatoria* spp., detritus and *Gammarus lacustris* biomass were determined bimonthly during 1991 at ten cobble–riffle sites in the dam-controlled Colorado River from Lees Ferry [river kilometer (RK) = 0.0] to Diamond Creek (RK 362; Stevens, 1983; Blinn *et al.*, 1992). Three replicated samples were taken at each site with a Hess sampler (1000 μm mesh), sorted live within 24 h of collection and oven-dried at 60°C to constant mass. An ash-free dry weight (AFDW) conversion was determined for *Cladophora* by ashing sixty samples for 1 h at 500°C and reweighing:

AFDW = 0.34825 (dry wt) + 0.04912
($R^2 = 0.923$; $F_{1,58} = 707.79$, $P < 0.001$).

All *Oscillatoria* samples were ashed and are reported as AFDW.

The abundance of *G. lacustris* within tufts of *C. glomerata* with and without the associated diatom community was investigated at Lees Ferry, Arizona. Ten 150 g tufts of *C. glomerata* with diatoms, and ten 150 g tufts of *C. glomerata* without diatoms, were randomly collected and examined for *G. lacustris*. Diatom composition and density were determined with a Sedgwick–Rafter chamber.

Field experiments

Field experiments were conducted at Lees Ferry, Arizona to test the choice by *G. lacustris* among four common substrata in the Colorado River ecosystem. These substrata were *C. glomerata, Oscillatoria* spp., detritus and gravel. Round galvanized pans (100 cm^2 and 10 cm deep) were divided into four equal sections with 3 cm hard plastic tubing secured to the bottom of the pan with sheet metal screws and sealed with silicone caulk (Fig. 1). A groove was cut into each section of tubing and a 1.5 cm screen mesh 10 cm high was placed between sections to reduce lateral movement. These mesh 'fences' were 5 cm below the rim of the pan so amphipods could choose freely between substrata. Water flow through the pan was enhanced with three side holes (3 cm diameter) drilled in each section. The entire pan was covered with 1 mm nylon mesh secured with large rubber bands. A random block design was used for the arrangement of substrata in each pan. Three blocks of eight pans ($n = 24$) were placed in shallow (<60 cm)

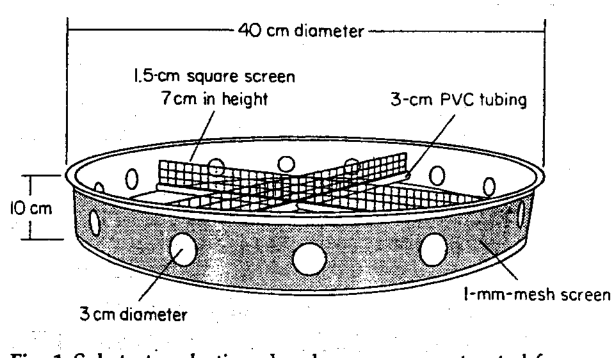

Fig. 1 Substrata selection chambers were constructed from galvanized pans (100 cm^2) that were divided into four sections. Section fences were designed to keep substratum in place but allow movement of animals between sections. A plate of iron (20 × 20 cm; 1 kg) was bolted to the bottom for ballast.

water at Lees Ferry ($<0.2\,m\,s^{-1}$). Each block was 2 m apart and every pan was 1 m apart.

Cladophora glomerata and *Oscillatoria* were collected from a cobble bar at Lees Ferry and detritus (0.5–5.0 cm in diameter) was gathered from the Paria River, 1.2 km below Lees Ferry. Detritus consisted of woody material, allochthonous in origin. Gravel (0.5–1.5 cm diameter) was collected at Lees Ferry and sterilized. Macroinvertebrates were removed from algal and detrital substrata and an approximately uniform volume of each substratum ($90\,cm^3$) was used for each category.

Substrates were randomly placed into sections of each pan, so that within a block no two pans were identical in configuration (Fig. 1). Twenty-four amphipods (15–20 mm) were placed into each experimental pan. This number of amphipods was within 1 SD of the annual mean density of *G. lacustris* found in *C. glomerata* at Lees Ferry ($n = 6$, $df = 18$) (Blinn *et al.*, 1992). Experiments were conducted for 1 h at 12.00, 15.00 and 19.00 h *in situ* at Lees Ferry to compare light and dark treatments. Amphipods were released in the centre of treatment pans for 12.00 and 19.00 h experiments, while animals were released in randomly chosen habitats in the 15.00 h experiment. Amphipod densities per substrate were determined immediately after each treatment.

Laboratory experiments

Laboratory substratum choice experiments were conducted in plastic containers ($30 \times 12 \times 12$ cm) with 1.5 l of filtered river water. Each container was divided into three sections with 1 cm mesh screen caulked into place. *Gammarus lacustris* was given a paired choice; substratum types were placed at each end of the containers, with no substratum in the centre section. The chambers were incubated at $50\,\mu Ein\,m^{-2}\,s^{-1}$ for 4 and 8 h at 10°C, the mean annual temperature of the Colorado River (Blinn *et al.*, 1992). *Cladophora glomerata* with diatoms, *C. glomerata* with few diatoms, and amphipods were collected at the Lees Ferry cobble bar (0.8 km). All macroinvertebrates were removed from *C. glomerata* (150 g wet wt) before placing into chambers. Each experiment was scored as *C. glomerata*, string and centre (no preference).

Three habitat choice experiments were conducted in the laboratory with *G. lacustris*. In the first experiment, *G. lacustris* was given a choice of *C. glomerata* with associated diatoms and *C. glomerata* which had been sonicated (Bransonic 35) in river water for 5 min to remove epiphytic diatoms. Microscopic observations indicated that this procedure removed at least 80% of the epiphytic diatoms. Two trials were run with six containers and fifteen amphipods and another two trials were run with five containers. Both sets of trials were examined after 4 and 8 h under light and dark conditions.

In a second laboratory experiment, *G. lacustris* was given a choice of string soaked for 24 h in epiphytic diatom extract and string soaked in filtered river water. Cotton string (15 g dry wt), 1 mm diameter, was used to simulate the filamentous structure of *C. glomerata*. Two trials with each string treatment were run with five containers and fifteen amphipods and two trials were run with eight containers and twenty-five amphipods. Both sets of trials were scored after 4 and 8 h under light and dark conditions.

In a third experiment, *G. lacustris* was offered the choice of *C. glomerata* with diatoms and string soaked in filtered river water. This trial tested for the effect of string. Sets of twenty-five amphipods were placed into eight containers for two trials and scored at 4 and 8 h under light and dark conditions.

Statistical analyses

Multiple regression analyses were used to analyse field collection data to determine the relationship between *C. glomerata*, *Oscillatoria* spp., detritus and *G. lacustris* biomass. The Friedman random block design test was used to analyse field habitat selection experiments with predictor variables of *C. glomerata*, *Oscillatoria*, detritus and gravel. *G. lacustris* density was the response variable. These data were also analysed by ANCOVA with depth and current velocity as covariates. A *post-hoc* HSD Tukey test was used to determine differences between treatments. Laboratory substratum selection experiments were analysed using the Kruskal–Wallace non-parametric test, with substratum types as the predictor variables and *G. lacustris* density the response variable. MANOVA was used to determine the effect of light and duration of experimental runs on *G. lacustris* substratum preference. All calculations were performed with SYSTAT computer software (Version 5.1; Wilkinson, 1989).

Results

Although highly variable, biomass of *Gammarus lacustris* was significantly ($F_{3,397} = 103.5$, $P < 0.001$) correlated with biomass of *Cladophora glomerata* in the Colorado River between Lees Ferry and Diamond Creek (Fig. 2). In contrast, *G. lacustris* biomass showed no correlation with *Oscillatoria* biomass ($F_{3,397} = 0.19$, $P = 0.38$), or detritus ($F_{3,397} = 0.76$, $P = 0.65$), both of which were common below the confluence of the Paria River.

Field observations at Lees Ferry indicated significantly higher numbers of *G. lacustris* in tufts of *C. glomerata* with epiphytic diatoms (brown in colour) than in *C. glomerata* with reduced densities (bright green in colour) of epiphytic diatoms ($F_{1,19} = 11.361$, $P = 0.003$). *C. glomerata* with epiphytes had three times more amphipods than *C. glomerata* with reduced epiphytic diatoms. Average cell density of epiphytic diatoms on *C. glomerata* was 14 400 cells per 150 g *C. glomerata* (wet wt), whereas average epiphyte density on *C. glomerata* with reduced epiphytes was less than 2500 cells per 150 g *C. glomerata*. The composition of the epiphytic diatom community of *C. glomerata* was dominated by *Diatoma vulgare*, *D. tenue* and *Fragilaria* spp.

Results of the habitat choice experiments conducted at Lees Ferry also indicated a positive relationship between *G. lacustris* and *C. glomerata*. *Cladophora glomerata* was selected by *G. lacustris* over *Oscillatoria*, detritus and gravel (Fig. 3). Application of the Friedman non-parametric test (145.7, $P < 0.0001$) resulted in the following rank sums: *C. glomerata* = 283, *Oscillatoria* = 193, detritus = 137.5, and sterile gravel (control) = 110. Significant differences in *G. lacustris* abundance were found between all habitat pairs (Tukey HSD $P < 0.0001$) except for detritus and gravel. No significant difference occurred between experiments ($F_{2,285} = 1.08$, $P = 0.33$), so all *in situ* experiments were pooled in making these conclusions. Therefore, time of day or light conditions are not factors in *G. lacustris* substratum preference. Also, depth and current velocity did not alter habitat choice by *G. lacustris* over a range in depth of 10–37 cm and current velocities of 0–0.18 ms^{-1} (Table 1).

Laboratory choice experiments provided evidence that epiphytic diatoms play an important role in the positive relationship between *G. lacustris* and *C. glomerata* in the Colorado River. *G. lacustris* selected *C. glomerata* with epiphytic diatoms twice as often as sonicated *C. glomerata*, with few epiphytic diatoms ($F_{2,39} = 218.16$, $P < 0.0001$). *G. lacustris* also selected string soaked in diatom extract three times more often then plain string ($F_{2,16} = 25.7$, $P < 0.001$), sug-

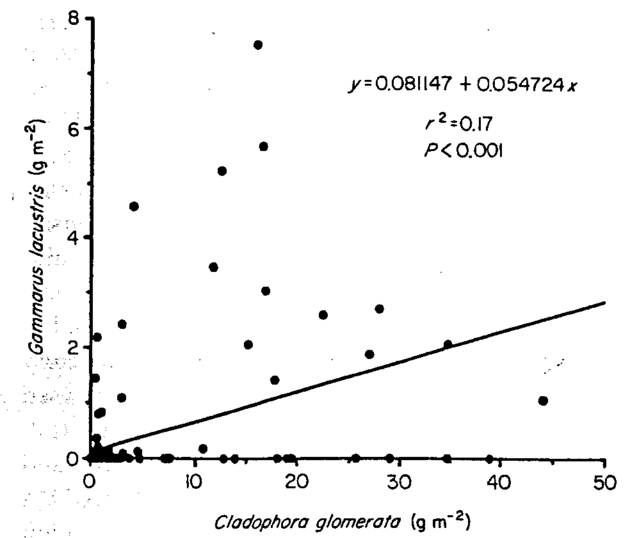

Fig. 2 Regression analysis of *Gammarus lacustris* and *Cladophora glomerata* in the Colorado River through Grand Canyon. *C. glomerata* has a positive correlation for *G. lacustris* distribution on cobble/riffle habitats. The low regression value can be explained by the patchy distribution of *C. glomerata* with and without epiphytic diatoms. $n = 397$.

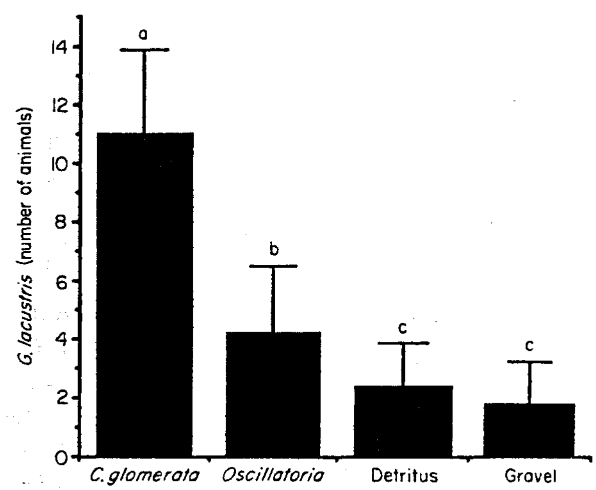

Fig. 3 Results of *in situ* habitat selection experiment with *Gammarus lacustris* at Lees Ferry, Arizona. The abscissa represents mean number of amphipods that selected various substrata. *Cladophora glomerata* was the preferred habitat over *Oscillatoria* spp. and detritus; ANOVA analysis ($F = 276.47$; $P < 0.0001$; $n = 72$; ±SD).

Table 1 ANCOVA analysis of the *in situ Gammarus lacustris* habitat selection experiment. Depth and current did not significantly affect habitat selection. *Cladophora glomerata* was significantly preferred as substratum

Source	Sum of squares	df	Mean square	F	P
Habitat	2228.3	3	742.7	139.6	<0.00001
Depth	0.1	1	0.1	0.02	0.887
Current	515.8	1	1.68	0.31	0.575

gesting that epiphytic diatoms provide a strong attraction for *G. lacustris*. Finally *G. lacustris* selected *C. glomerata* with diatoms three times more frequently than extract-free string ($F_{2,60} = 39.4$, $P < 0.0001$).

Discussion

Although variable, the distribution of *Gammarus lacustris* was positively correlated with the distribution of the green filamentous alga, *Cladophora glomerata*, and its epiphytic diatom assemblage in the Colorado River through Grand Canyon. *In situ* habitat choice experiments supported these findings: *G. lacustris* chose *C. glomerata* over *Oscillatoria* sp., detritus or gravel in a 3:1:1:1 ratio, respectively. The *Oscillatoria* spp. assemblage in the Colorado River included relatively few epiphytic diatoms compared with the dense diatom assemblage associated with *G. glomerata* (Blinn *et al.*, 1992).

The insignificant variation in substratum preference between light and dark treatments for *G. lacustris* was unexpected. *Gammarus* is a common component of drift (Marchant & Hynes, 1981) that reaches maximum drift density (Brittain & Eikeland, 1988) and drift distance (Elliott, 1971) at night. An inference from these reports would be that more dispersal among the substratum would be expected during dark treatments, especially for *in situ* experiments. Perhaps the need by *G. lacustris* to enter the water column in search of food is reduced due to the abundant epiphytic diatoms (Blinn *et al.*, 1992).

The overriding importance of epiphytic diatoms on *C. glomerata* (as food) was demonstrated in both field observations and laboratory choice experiments. *G. lacustris* showed a significant preference for tufts of *C. glomerata* with epiphytic diatoms over *C. glomerata* tufts of similar size with few epiphytic diatoms. Also, *G. lacustris* preferred string soaked in diatom extract over string soaked in Colorado River water. Over 95% of the diet of *G. lacustris* at Lees Ferry is composed of epiphytic diatoms associated with *C. glomerata*; less than 1% of the diet is composed of *C. glomerata* (Pinney, 1991). Patrick *et al.* (1983) suggested that *Cladophora* may not be readily consumed by macroinvertebrates because it is a relatively poor food source. However, Dodds & Gudder (1992) report that several freshwater invertebrates consume *Cladophora*.

Our data indicate that epiphytic diatoms may provide chemical cues for *G. lacustris*. The epiphytic diatom assemblage chemically attracts the herbivore (*G. lacustris*) into the *C. glomerata* assemblage.

The rather weak association between *G. lacustris* and *C. glomerata* in the field as opposed to the laboratory is probably a consequence of complex and variable interactions among substratum (*Cladophora*), food (epiphytic diatoms), and *G. lacustris* within the Colorado River. In particular, *C. glomerata* tufts with high densities of epiphytic diatoms are likely to show stronger positive associations with *G. lacustris*, than tufts with low epiphytic densities.

The high standing crop of *C. glomerata* in the tailwater reach is due, in part, to abundant armoured substrata and the hypolimnetic release of constantly cold, clear water from Glen Canyon Dam (Stanford & Ward, 1991). The decrease in standing stock of *G. lacustris* with distance from Glen Canyon Dam (Blinn & Cole, 1991; Blinn *et al.*, 1992) corresponds to a decrease in *C. glomerata* biomass and associated epiphytic diatoms downriver (Hardwick, Blinn & Usher, 1992). The decrease and variable distribution of both *C. glomerata* and associated epiphytic diatoms have been attributed to an increase in suspended sediment load below two major tributaries in the Grand Canyon (Paria River 26 km below the dam, and the Little Colorado River, 123 km below the dam) (Andrews, 1991), and to river regulation (Usher & Blinn, 1990; Blinn *et al.*, 1992; Hardwick *et al.*, 1992).

Whereas some marine amphipods select algal substrata for protection from predators (Duffy & Hay, 1991), *G. lacustris* may be exposed to greater predation by selecting *C. glomerata* as a substratum. Rainbow trout (Leibfried, 1988) and waterfowl (Stevens & Kline, 1991) readily consume *C. glomerata* and associated amphipods in the Colorado River. Therefore, in contrast to some marine associations (Hay *et al.*, 1987), the *Gammarus*/*Cladophora* association in the Colorado River appears to be driven primarily by food. As noted by Bell (1991), there are also important amphipod/epiphyte linkages in marine ecosystems. In the Colorado River we consider *C. glomerata* to be the architectural host plant that provides a substratum for colonization by epiphytic diatoms, the food source.

This system generally conforms to Kogan's (1977) fifth model of chemical influences on terrestrial plant/herbivore interaction in which host plant chemical cues are used by the herbivore as stimulants for host location and subsequent feeding or oviposition. For example, Cruciferae-feeding terrestrial Lepidoptera, Coleoptera and Diptera use mustard oils as cues to locate their host plants (Kogan, 1977).

Examination of interactions between *C. glomerata*, associated diatom epiphytes, and herbivores may reveal a mutualistic community. Epiphytes growing on *C. glomerata* attract herbivores. These herbivores, in turn, consume epiphytes and detritus trapped by epiphytic diatoms, alter the light climate, and release mineralized nutrients, which may be assimilated by the host plant (Dudley, Copper & Hemphill, 1986; Dodds, 1991b; Dodds & Gudder, 1992). Positive mutualistic interactions between marine macroalgae and amphipods have been documented by Brawley & Adey (1981) and Duffy (1990). However, the potentially mutualistic interaction between *C. glomerata*, epiphytes and *G. lacustris* in freshwater ecosystems requires further research (Dodds & Gudder, 1992). This form of mutualism is based on nutrient or food supply, and may be an important factor influencing aquatic benthic community structure.

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References

Andrews E.D. (1991) Sediment transport in the Colorado River basin. *Colorado River Ecology and Dam Management* (Ed. National Research Council), pp. 54–74. National Academy Press, Washington, DC.

Barlocher F. & Kendrick B. (1973) Fungi and food preference of *Gammarus pseudolimnaeus*. *Archiv für Hydrobiologie*, **72**, 501–516.

Bell S.S. (1991) Amphipods as insect equivalents? An alternative view. *Ecology*, **72**, 350–354.

Blinn D.W. & Cole G.A. (1991) Algae and invertebrate biota in the Colorado River: Comparison of pre- and post-dam conditions. *Colorado River Ecology and Dam Management* (Ed. National Research Council), pp. 85–104. National Academy Press, Washington, DC.

Blinn D.W. & Johnson D.B. (1982) Filter-feeding of *Hyalella montezuma*, an unusual behavior for a freshwater amphipod. *Freshwater Invertebrate Biology*, **1**, 48–52.

Blinn D.W., Stevens L.E. & Shannon J.S. (1992) *The effects of Glen Canyon Dam on the aquatic food base in the Colorado River Corridor in Grand Canyon, Arizona*. Bureau of Reclamation, Glen Canyon Environmental Studies. Report # GCES II-02, Salt Lake City, UT.

Brawley S.H. & Adey W.D. (1981) The effects of micrograzers on algal community structure in a coral reef. *Marine Biology*, **36**, 167–177.

Brittain J.E. & Eikeland T.J. (1988) Invertebrate drift—a review. *Hydrobiologia*, **166**, 77–93.

Deksbakh N.K. & Sokolova G.A. (1965) Biology of *Gammarus lacustris* Sars in some lakes of the central Urals (feeding). *Trudy sverdlosk sel-hoz Institute*, **12**, 475–80.

Dodds W.K. (1991a) Factors associated with dominance of the filamentous green alga *Cladophora glomerata*. *Water Research*, **25**, 1325–1332.

Dodds W.K. (1991b) Community interactions between the filamentous alga *Cladophora glomerata* (L.) Küetzing, its epiphytes, and epiphyte grazers. *Oceologia*, **85**, 572–580.

Dodds W.K. & Gudder D.A. (1992) The ecology of *Cladophora*. *Journal of Phycology*, **28**, 415–427.

Dudley T.L., Copper S.C. & Hemphill N. (1986) Effects of macroalgae on a stream invertebrate community. *Journal of the North American Benthological Society*, **5**, 93–106.

Duffy J.E. (1990) Amphipods on seaweeds: partners or pests? *Oecologia*, **83**, 267–276.

Duffy J.E. & Hay M.E. (1991) Food and shelter as determinants of food choice by an herbivorous marine amphipod. *Ecology*, **72**, 1286–1289.

Elliott J.M. (1971) The distances travelled by drifting